L10: Entry 36 of 37 File: USPT

DOCUMENT-IDENTIFIER: US 5362641 A
TITLE: Heparanase derived from human Sk-Hep-1 cell line

Brief Summary Text (6):
Proteoglycans are high molecular weight compounds with a protein backbone; linked to the backbone are a number of side chains of different types of heteropolysaccharides. A high proportion of the molecular weight may thus be attributed to carbohydrates. These compounds can routinely be broken down by a number of different enzymes. The degradation of the proteoglycans usually begins with the proteolytic cleavage of the backbone to produce peptide components and glycosaminoglycans. The latter are in turn hydrolysable into smaller glycosaminoglycan fragments by endoglycosidase enzymes, and these are further degradable into monosaccharides by exoglycosidases. Heparan sulfate proteoglycans have an intimate interrelationship with the other macromolecules which make up the extracellular matrix, and thus, their degradation may have a profound effect on the regulation of cell anchorage, movement, function and growth.

Brief Summary Text (8):
A number of normal and abnormal physiological conditions and disorders have now been shown to be associated with the degradation of the extracellular matrix of various tissues. For example, neutrophil mobilization, as part of the inflammatory process, requires that the cells penetrate the endothelium of blood vessels and the underlying basal lamina in order to reach the target tissue. The penetration requires the specific and mild action of readily released enzymes (i.e., heparanase) expressed by the neutrophils under conditions which maintain the integrity of the vessel wall (Matzner et al., J. Clin. Invest. 76:1306-1313, 1985). Similarly, in the process of metastasis, tumor cells must invade the basal lamina of the vascular endothelium in order to be transported to other sites in the body. T-lymphocytes as well, in responding to the presence of an antigen, will penetrate the walls of blood vessels and subendothelial extracellular matrix. Cell invasion is typically achieved by enzymatic breakdown of the matrix surrounding the cells. The invading cells must, therefore, be capable of producing ECM degrading enzymes whenever necessary to achieve penetration of the wall of the blood vessel or other target tissue.

Brief Summary Text (9):
Since a number of proteoglycans are known to exist as part of the connective tissue, the degrading enzyme could theoretically be any one of a number of enzymes which attack a particular sulfated proteoglycan. For example, depending on the specific tissue involved, the ECM may contain chondroitin sulfate, dermatan sulfate, hyaluronate, keratan sulfate or heparan sulfate, in a characteristic combination. Each of these can be broken down by, respectively, chondroitinase, hyaluronidase, keratanase or heparanase. The ability of a given cell to penetrate a particular tissue type, then, is dependent to a large extent in the production, by the cell, of an enzyme or enzymes which can degrade the proteoglycans in the tissue. HSPG interact with various macromolecules in the ECM, such as collagens, laminin and fibronectin. This suggests a key role for this proteoglycan in the structural integrity, self-assembly and insolubility of the ECM. Because of this property and because HSPG is the major proteoglycan of basement membranes, its degradation is necessary to allow penetration of cells through blood-vessels and epithelial basement membranes.

Brief Summary Text (10):
It has recently been observed that a certain type of endoglycosidase is produced by particular kinds of cells. For example, migrating human neutrophils have been shown to degrade heparan sulfate by secretion of heparanase, (Matzner, et al., supra). The same

type of enzyme has been shown to be produced by metastatic melanoma cells (Nakajima et al., J. Biol. Chem. 259:2283-2290, 1984; Vlodavsky et al., Exptl. Cell Res. 140:149-159, 1982) and metastatic lymphoma cells (Vlodavsky et al., Cancer Res. 43:2704-2711, 1983). Antigen-stimulated T-lymphocytes also secrete heparanase, shortly after the exposure to antigen (Naparstek et al., Nature 310:241-243, 1984; Fridman et al., J. Cell. Physiol. 130:85-92, 1987). The endoglycosidase heparanase thus appears to play an integral role in a number of specific physiological functions, such as tumor metastasis (by way of aiding in invasion of blood vessels, basement membranes, and ECM) and autoimmune disorders (by aiding in extravasation of activated cells in the immune system). The use of heparanase inhibitors in impeding tumor metastases and autoimmune disorders has previously been suggested (WO 88/01280, 1988).

Brief Summary Text (11):
Biochemical analysis of HS degradation products revealed that the melanoma cell heparanase cleaves .beta.-D-glucuronosyl-N-acetylglucosaminyl linkages in HS (Nakajima et al., J. Biol. Chem. 259:2283-2290, 1984). The melanoma and liver endo-.beta.-D-glucuronidases appear to degrade HS but not heparin. In contrast, the human platelet enzyme depolymerizes both HS and heparin and an endoglucuronidase from a mouse mastocytoma exhibits strict specificity towards macromolecular heparin proteoglycan.

Brief Summary Text (13):
In addition to the functions noted above for heparanase, it has also been noted that this enzyme has the effect of causing release of angiogenic endothelial cell growth factors from basement membranes and subendothelial ECM (Vlodavsky et al., PNAS USA 8.4:2292-2296, 1987; Folkman et al., Am. J. Pathol. 130:393-400, 1988; Bashkin & Vlodavsky, Biochemistry, 2.8:1737-1743, 1989. "Angiogenesis" refers to the process of formation of new blood vessels, which accompanies a number of normal and abnormal physiological processes in the body. For example, angiogenesis typically accompanies the process of tumor formation, as well as the process of tissue repair in wound healing. Recently, a number of angiogenic factors have been discovered to occur naturally in many tissues of the body (Folkman and Klagsbrun, Science 235:442-447, 1987). Angiogenin, for example, is a polypeptide shown to have angiogenic activity in a rabbit cornea and chick embryo. Its mode of activity is uncertain, but it has been suggested that it causes release of endothelial mitogens or chemoattractants from host cells, or mobilizes macrophages to release these factors. Transforming growth factors (TGF) are also polypeptides which alter the behavior of fibroblasts and other cells in culture, and exhibit angiogenic activity in vivo (Folkman and Klagsbrun, supra). Other factors, such as low molecular weight endothelial mitogens, endothelial cell chemotactic factors, and certain lipids, such as prostaglandins, have been shown to be angiogenic but, as yet, their mode of activity is poorly characterized. None of these aforementioned factors have been shown to have any relationship to heparanase.

Brief Summary Text (15):
Another group of factors, known as fibroblast growth factors (FGF) and endothelial cell growth factors (ECGF) are distinguished from these other angiogenic compounds by their ability to bind heparin with high affinity (Lobb et al. J. Biol. Chem. 261:1924-1928, 1986). Heparin is a well known compound which has been found to be circumstantially associated with angiogenesis in many different situations, but its direct role in the process is as yet unclear. However, these heparin-binding growth factors have been more directly implicated in the process. These factors were among the first to be so identified; FGF encompasses two classes of polypeptides, cationic or basic FGF, and anionic or acidic FGF, with ECGF being an extended form of acidic FGF. Both types of heparin-binding growth factors have been shown to stimulate endothelial cell migration and growth both in vitro and in vivo, and also appear to induce the formation of highly vascularized granulation tissue (Shing et al., J. Cell. Biochem. 29: 275-287, 1985).

Brief Summary Text (18):
In addition to its role in angiogenesis and stimulation of endothelial cell migration and growth, FGF, as its name implies, also is essential in the proliferation of fibroblasts and virtually all other mesoderm and neuroectoderm-derived cells. Fibroblasts are mesenchymal connective tissue cells which are responsible for the production of collagen fibers, one of the major building blocks of connective tissue. Both the endothelial and fibroblast proliferation are essential elements of the

process of wound healing. When an injury occurs, a number of events, involving many different cell types, occur shortly thereafter. White blood cells, particularly phagocytic white blood cells, are rapidly attracted to the site to clean the wound by removing foreign particles, including microbes. At the same time, fibroblasts appear in large numbers in the area, and begin laying down collagen, in the process of preparation and replenishment of connective tissue. The region is also invaded by endothelial cells, forming new capillaries to supply the new tissue with nutrients and to remove waste from the area. Eventually, the damaged tissue is completely replaced by new tissue and epithelial cells migrate from all sides to eventually cover the surface. Poorly healing wounds, or non-healing wounds (ulcers), occur in many clinical settings, including atherosclerosis and diabetes; as side effects of treatments associated with steroid therapy, immunotherapy, radiotherapy and chemotherapy; in pressure sores; and as a result of a variety of injuries in the elderly. Burns represent a special kind of non-healing wounds. While the commonest cause of poor healing is infection, other major factors include failure to recruit mesenchymal cells and failure to develop and maintain an adequate blood supply. For this reason, mitogens found to attract and promote proliferation of mesenchymal cells in vitro and to induce neovascularization in vivo, are under intensive study as wound healing agents (Lobb, Euro. J. Clin. Invest. 18:321-326, 1988).

Brief Summary Text (19):
Given an understanding of the mechanisms involved in wound healing, it appears that FGF may play a significant role in the process, by way of its effect on angiogenesis, as well as its effect on fibroblast growth. The ability to control its release, or to stimulate its production, could have profound effects on improving the wound healing process. However, at this time no known method has effectively utilized FGF in enhancing the healing process. Although recombinant FGF is known, it resembles various human oncogene products (Thomas, TIBS 13:327-328, 1988) and may, in some circumstances, initiate cell transformation (Rogelj et al., Nature 331:173-175, 1988). High doses of FGF have also been shown to be toxic to various cell types, including endothelial cells.

Brief Summary Text (20):
As noted above, the FGF is bound in situ in the extracellular matrix (ECM) to heparan sulfate, and can be released by the addition of heparanase to the ECM. Addition of heparanase may thus provide an effective method to mobilize and activate the ECM-bound FGF and hence to promote the wound healing process. The use of heparanase to release FGF from its natural setting has the advantage of the cells' responding locally to the endogenous natural growth factor and in appropriate amount. However, the preparations of heparanase that are currently known are too crude to be used therapeutically in displacing FGF from the connective tissue in which it is bound. A relatively pure heparanase is required before this enzyme can be contemplated for use in vivo in humans. Pathological conditions other than wound healing, which are likely to benefit from neovascularization promoted by FGF include cardiac, cerebral and peripheral ischaemic diseases and diseases associated with vascular damage, such as diabetes, hypertension and systemic lupus erythematosus. Other potential clinical applications for angiogenic factors are in processes such as ovulation, hair growth, transplantation, nerve regeneration and bone and cartilage repair.

Brief Summary Text (23):
The heparanase so produced has a purity of at least 4000-fold over the crude cell extract. This purified heparanase provides the basis for useful pharmaceutical compositions, comprising the purified heparanase in combination with a pharmaceutically acceptable, preferably slow releasing, carrier. Such a composition is useful for the treatment of wounds, and enhancement of the wound-healing process. It is also useful for the treatment of any other physiological state or condition in which neovascularization or angiogenesis would be expected to be of benefit. Wound treatment can be achieved by administration to an afflicted individual an effective amount of the composition of the present invention .

Drawing Description Text (5):
FIGS. 3A(A), 3A(B) and 3A(C): Release of ECM-bound FGF by various purified ECM degrading enzymes. FIG. 3A(A): ECM-coated wells were incubated (90 min, 22.degree. C.) with .sup.125 I-bFGF, washed and treated with heparanase ( ), heparinase (o), chondroitinase ABC ( ), or chondroitinase AC ( ). Radioactivity released into the

incubation medium is expressed as % of total ECM-bound .sup.125 I-FGF. "Spontaneous" release in the presence of buffer alone was 5%-7% of the total ECM-bound FGF; FIG. 3A(B) ECM-coated 4-well plates were incubated (90 min, 22.degree. C.) with bFGF, washed and treated (0.1 units/ml, 60 min, 37.degree. C.) with heparanase ( ), heparinase (o), chondroitinase ABC ( ), hyaluronidase (.DELTA.), collagenase ( ) or trypsin ( ). Aliquots (20 ul) of the incubation medium were tested for stimulation of DNA synthesis in growth-arrested 3T3 cells; FIG. 3A(C) ECM-coated 4-well plates were untreated ( ) or pretreated with heparanase (0.1 u/ml, 60 min, 37.degree. C.) ( ) or chondroitinase ABC (0.5 u/ml, 60 min, 37.degree. C.) ( ). ECM was washed, incubated (90 min, 22.degree. C.) with .sup.125 I-FGF, and the amount of ECM-bound FGF was determined.

Detailed Description Text (4):
Heparanase has been found to occur in many different cell types, e.g., metastatic melanoma cells (Vlodavsky et al., Exptl. Cell Res. 140:149-159, 1982; Nakajima et al., Science 220:611-612, 1983), neutrophils (Matzner, J. Clin Invest. 76: 1306-1313, 1985), T-lymphocytes (Naparstek et al., Nature 310:241-243, 1984; Fridman et al., J. Cell Phys. 130:85-92, 1987), or metastatic lymphoma cells (Vlodavsky et al., Cancer Res. 43:2704-2711, 1983). Extracts of any of these cell types can be used as a starting material for purification of heparanase. A cell line which produces significant amounts of heparanase in culture is the human hepatoma Sk-Hep-1 cells. (Klagsbrun et al., PNAS USA 83:2448-2452, 1986). The cell line Sk-Hep-1 is publicly available from the American Type Culture Collection as accession number HTB 52. The enzyme is secreted in relatively small amounts into the medium, so preparation of cell lysates is generally preferred. A crude heparanase extract may be obtained by homogenization of freezed and thawed cultured cells, centrifugation to pellet out the cell debris and subsequent collection of the supernatant.

Detailed Description Text (6):
A platelet endo-.beta.-D-glucuronidase has been partially purified by Oosta et al. (J. Biol. Chem. 257:11249, 1982). This enzyme is capable of degrading both HS and heparin and has a Mr of 134,000. Heparanase (Mr.about.96,000 dal) has also been partially reportedly purified from mouse B16-F10 melanoma cells by use of heparin-Sepharose, Con-A Sepharose and N-acetylated, N-desulfated heparin-Sepharose (Nakajima et al., J. Cell. Biochem. 36:157-167, 1988, purification mentioned but not described).

Detailed Description Text (8):
The purified heparanase, and the formulations containing same, may be used in therapy, in situations in which release of FGF from the extracellular matrix would be a desirable effect. More specifically, the heparanase formulations are useful in situations in which angiogenesis and/or the growth of fibroblasts is desired. A common situation in which the preparations would be useful for this purpose is for the stimulation and enhancement of wound healing.

Detailed Description Text (11):
The utility of the purified heparanase is not limited to its therapeutic use. The availability of a purified enzyme provides a basis for preparation of specific anti-heparanase polyclonal and/or monoclonal antibodies. Availability of such antibodies will enable detection, quantitation and localization of heparanase in blood samples, body fluids, pleural efflusions and biopsy specimens for diagnostic purposes. Such antibodies were, in fact, prepared in our laboratory against active heparanase eluted from a native polyacrylamide gel. These antibodies have been used for detection of heparanase in column fractions and biopsy specimens (FIGS. 5AII(A) and 5AII(B)).

Detailed Description Text (16):
Cultures of bovine corneal endothelial cells were established from steer eyes as previously described (Vlodavsky et al., Cancer Res. 43:2704-2711, 1983). Stock cultures were maintained in DMEM (1 g glucose/liter) supplemented with 10% bovine calf serum, 5% FCS, penicillin (50 u/ml) and streptomycin (50 .mu.g/ml) at 37.degree. C. in 10% Co.sub.2 humidified incubators. Partially purified brain-derived bFGF (100 ng/ml) was added every other day during the phase of active cell growth. Bovine aortic and capillary endothelial cells were cultured as described (Vlodavsky et al., PNAS USA 84:2292-2296, 1987).

Detailed Description Text (19):

Culture dishes coated with sulfate-labelled ECM were prepared as described above, except that Na.sub.2 [.sup.35 S]O.sub.4 (540 mCi/mmole) was added twice (40 uCi/ml) into the culture medium on the 3rd day after seeding, when the cells were nearly confluent, and 4 days afterward. The cultures were incubated with the labelled sulfate with no medium change and 10-12 days after seeding, the cell layer was dissolved and ECM prepared, as described (Vlodavsky et al., Cancer Res., supra; Naparstek et al., Nature 310:241-243, 1984). 75%-85% of the ECM radioactivity was incorporated into HS.

Detailed Description Text (21):
Heparan sulfate (HS) was isolated from unlabelled and sulfate-labelled ECM using methods described for isolation of HS from bovine aorta (Schmidt et al., Eur. J. Biochem 125:95-101, 1982) and swarm sarcoma basement membrane (Hassell et al., PNAS USA 77:4494-4498, 1980). For isolation of total glycosaminoglycans (GAGs), either labelled or unlabelled ECM is digested in 0.05M acetate buffer (pH 4.5) containing 0.5% papain, 0.05% EDTA and 0.005M cysteine at 65.degree. C. for 24 hours, followed by alkaline degradation in 0.15% KOH for 4 hours at 37.degree. C. and precipitation by cetylpyridinium chloride (CPC). The pellet containing mostly HS, dermatan sulfate and chondroitin sulfate is digested with 0.8 mU chondroitinase ABC in 10 ml 0.5M Tris buffer, pH 8.0, at 37.degree. C. for 24 hours. A further 0.8 mU is added after the first 12 hours. The digest is precipitated with ethanol at a final concentration of 50% in the presence of 5% calcium acetate and 0.5M acetic acid. The precipitate is dissolved in and dialyzed against distilled water and reprecipitated several times with ethanol. Purification of HS is carried out by ion exchange chromatography on Dowex 1 X2 (200-400 mesh, chloride form) and elution with a continuous sodium chloride gradient (0.1-2.0M NaCl). HS is eluted at about 1.0M sodium chloride. HS containing fractions are pooled, exhaustively dialyzed against distilled water, and precipitated by two volumes of ethanol containing 1% potassium acetate (Schmidt et al., supra).

Detailed Description Text (24):
Cells, conditioned medium, or cell-free enzyme preparations are incubated (4-24 hours, 37.degree. C., pH 6.0-6.5) with sulfate-labelled ECM. The reaction mixture is centrifuged (10,000 g, 5 min) and 0.5 ml aliquots of the supernatant are applied for gel filtration on Sepharose 6B columns (0.7.times.35 cm) equilibrated with PBS. Fractions (0.2 ml) are collected at a flow rate of 5 ml/h, and counted for radioactivity. The excluded volume (V.sub.o) is marked by blue dextran, and the total included volume (V.sub.T) by phenol red. Similar gel filtration profiles (K.sub.av values) are obtained by using ECM produced by corneal or vascular EC. Recoveries of labelled material applied to columns ranged from 85% to 95% in different experiments. Elution positions (Kav) of heparanase-mediated degradation products ranged from 0.5 to 0.75, depending on the source of enzyme and incubation conditions, but for a given enzyme preparation the variation in Kav values did not exceed +/- 15% in different determinations.

Detailed Description Text (26):
Metabolically .sup.3 H-glucosamine- or Na.sub.2.sup.35 SO.sub.4 -labelled HS is isolated from bovine aorta or ECM, as described above. Labelled HS is coupled to AH-Sepharose (Pharmacia) with carbodiimide at pH 5.0 while gently stirring the reaction mixture at 4.degree. C. for 24 hours. To remove the excess of uncoupled ligand, the gel is washed alternately with 0.2M NaHCO.sub.3 buffer (pH 8.3) and 0.1M acetate buffer (pH 4) 4-5 times, each buffer containing 0.5M sodium chloride. The gel is washed several times with distilled water and stored at 4.degree. C. Labelled immobilized HS (100 .mu.l, 5.times.10.sup.3 cpm) is incubated (4-24 hours, 37.degree. C. with gentle agitation) with the test materials (cells, cell extracts, conditioned medium, purified heparanase) in a final volume of 0.5 ml PBS (pH 6.2) in the absence and presence of heparin (20 .mu.g/ml). The reaction mixture is then centrifuged and radiaoctivity in the pellet and supernatant determined separately. Radioactivity released in the presence of heparin represents non-specific release or cleavage by enzymes other than heparanase. We have also used the solid phase heparanase substrate developed by Nakajima et al. (Anal. Biochem. 157:162-171, 1986) and prepared by cross-linking of partially N-desulfated and N[.sup.14 C or .sup.3 H] acetylated HS onto agarose beads via one covalent linkage (id.). For this purpose, labelled HS is first aminated at the reducing terminal with 2M ammonium acetate in the presence of 0.4M sodium cyanoborohydride in 50% methanol at 50.degree. C. for 6 days. The aminated HS is then purified by gel filtration and incubated with 1 ml Affi-Gel. The coupling reaction is continued at 4.degree. C. for 48 hours, and the beads reacted with 0.1M

glycine monoethyl ester to remove noncovalently attached HS from the beads (id.).

Detailed Description Text (28):
We are currently using microtiter plates (96 wells) coated with sulfate-labelled ECM for screening of heparanase activity in large numbers of fractions eluted in the course of enzyme purification. Samples (50-200 .mu.l) are added to each well, the plate is incubated (4-12 hours) at 37.degree. C., and the amount of released radioactivity is indicative of heparanase activity in each fraction. We have used this screening assay while purifying the hepatoma heparanase, and verified that the released radioactivity constitutes mostly HS degradation products (0.5<Kav<0.75) when applied onto Sepharose 6B. It is important to emphasize that, whenever possible, and particularly in studies with intact cells, we are performing the time-consuming bioassay of ECM degradation involving gel filtration analysis of HS degradation products. Requirements for degradation of HS in a multimolecular structure such as ECM differ from heparanase-mediated degradation of soluble HS (Matzner et al., J. Clin. Invest. 76:1306-1313, 1985).

Detailed Description Text (44):
Incubation (24 hours, 37.degree. C., pH 6.2) of Sk-Hep-1 cells on sulfate-labelled ECM resulted in release of high Mr (approx. 0.5.times.10.sup.6 Da) and low Mr (5.times.10.sup.3 -1.times.10.sup.4 Da) labelled material which eluted from Sepharose 6B with Kav<0.2 (peak I) and 0.35<Kav<0.8 (peak II), respectively. A similar incubation of Sk-Hep-1 cell lysates with the labelled ECM resulted in release of mostly low Mr degradation products (Kav=0.72 on Sepharose 6B) (FIG. 4). These low Mr (approx. 8,000 dal) fragments were (i) 5-7 fold smaller than intact, ECM-derived HS side chains; (ii) resistant to digestion with papain or chondroitinase ABC; and (iii) sensitive to cleavage with nitrous acid--all characteristic features of an endoglycosidase (heparanase) produced HS degration fragments. About 90% of the released radioactivity was precipitated with 0.05% cetylpyridinium chloride in 0.6M NaCl, conditions that specifically precipitate heparan sulfate sequences. Furthermore, release of low Mr HS cleavage products was inhibited by heparin (FIG. 4), a potent inhibitor of heparanase-mediated HS degradation. These results indicate that heparanase is expressed by intact Sk-Hep-1 cells and that a much greater activity can be extracted from lysates of these cells. Medium conditioned by cultured Sk-Hep-1 cells contained only small amounts of heparanase activity. Hepatoma cell lysates were therefore used in subsequent experiments as a source for purification of the enzyme.

Detailed Description Text (46):
Sk-Hep-1 cells cultured in spinner flasks to a density of 10.sup.6 cells/ml were harvested by centrifugation (250 g.times.10 minutes) and washed twice in phosphate buffered saline, pH 7.4 (PBS). The pellet of 1-2.times.10.sup.10 cells was resuspended in 300 ml of 1M NaCl, 0.01M Tris-HCl, pH 7.5, and subjected to 3 cycles of freezing and thawing, followed by homogenization in a Waring Blender. The homogenates were centrifuged (20,000 g for 30 min), the supernatant dialyzed against 0.1M NaCl, 0.01M Tris-HCl, pH 7.0, and applied onto a Biorex 70 column (5.times.40 cm) for batch elution of the enzyme with 0.5M NaCl in 10 mM Tris pH 7.0. Active material was dialyzed against 10 mM phosphate citrate buffer, 20 mM NaCl, pH 6.0, centrifuged at 20,000 g for 30 minutes, and the supernatant applied onto a 6.times.20 cm CM-50 Sephadex column. Heparanase activity was eluted with a linear salt gradient (0.01M-1M NaCl in phosphate citrate buffer) at a concentration of 0.7-0.8M NaCl (5AII(A) and 5AII(B)). 50%-60% of the total protein applied onto the CM-Sephadex column did not bind to the column, and about 30% of the bound protein was eluted at a salt concentration lower than that which eluted the enzyme (Table Ia). For determination of heparanase activity in this and subsequent purification steps, samples (50 .mu.l) of column fractions were first incubated (0.5-3 hours, 37.degree. C., pH 6.0) with sulfate-labelled ECM in 96-well plates for measurements of released radioactivity. Heparanase activity in active fractions was then verified by gel filtration analysis of the released material on Sepharose 6B columns, as described.

Detailed Description Text (47):
Two approaches were applied for further purification of heparanase eluted from CM-Sephadex. In the first procedure, the enzyme was subjected to heparin-Sepharose affinity chromatography, followed by gel filtration on Sephadex G-100. The other procedure utilized Con A-Sepharose chromatography.

Detailed Description Text (48):
A 50 kDa type 1 plasminogen activator inhibitor (PAI-1) may be present as a contaminant with the heparanase. The presence of PAI-1 may be detected with PAI-1 antibodies that cross-react with the purified heparanase preparation in Western blot analysis. The following modification of the purification protocol separates the heparanase enzyme from PAI-1 protein. Active heparanase eluted from CM-Sephadex is rechromatographed on CM-Sephadex at pH 7.4 in the presence of 0.1% CHAPS. Most of the PAI-1 is eluted closely before the protein having heparanase activity.

Detailed Description Text (51):
Gel filtration analysis (Superose 12, TSK-200) of heparanase preparations eluted from CM-Sephadex indicated a MW for heparanase in the range of 50-60 kD. Sephadex G-100 SF was therefore chosen as an appropriate resin for further purification and determination of the enzyme MW. For this purpose, fractions eluted from heparin-Sepharose were dialyzed, lyophilized and suspended in 1-2 ml of 0.5M NaCl in 0.01M sodium acetate, pH 5.5. As demonstrated in FIG. 5CI, heparanase activity was eluted as a single peak in fractions corresponding to a MW of about 50 kD (fractions 33-35). Estimated fold purification and yield of enzyme are presented in Table Ia. Samples of fractions 31-38 were subjected to SDS PAGE and silver staining to further assess the enzyme MW and degree of purification. A band corresponding to the expected MW (approx. 50 kD) correlated with heparanase activity in each of the fractions. Fraction #34, which exhibited the highest specific activity, contained, in addition to the major putative heparanase band, a protein of a slightly higher MW, and few proteins of lower MW which could barely be detected (FIG. 5CII).

Detailed Description Text (54):
Preliminary studies indicated that the hepatoma heparanase binds to Con A-Sepharose and elutes with 0.2M alpha-methylmannopyranoside. A higher degree of binding specificity, resulting in a better purification of the enzyme, was obtained when 1M NaCl was included in the sample and elution solutions. For this purpose, active fractions eluted from CM-Sephadex were dialyzed against 1M NaCl containing 10 mM sodium acetate, pH 6.0, 1 mM MgCl.sub.2, 1 mM CaCl.sub.2, and 1 mM MnCl.sub.2. More than 95% of the total sample protein did not bind to the column under these conditions, and heparanase could not be detected in either the unbound or column wash material. In contrast, 80%-90% of the total enzyme activity was recovered in the alpha-methylmannoside eluate containing <2% of the total protein which was loaded on the column (FIG. 5DI, Table Ib). SDS PAGE revealed the presence of a major doublet protein in the 50 kD region, in addition to some lower MW proteins, including the 27 kD subunit of the Con A molecule itself (FIG. 5DII).

Detailed Description Text (55):
In order to obtain a single band preparation, enzyme eluted from Con A-Sepharose was subjected to native polyacrylamide gel electrophoresis, as described in Materials and Methods. The gel was cut into strips of 5 mm, and protein was electroeluted from 5 mm segments of each strip. Heparanase activity, measured by gel filtration analysis of sulfate-labelled degradation products, was eluted primarily from strip #7. A much lower activity was detected in strip #4 (FIG. 6). Enzyme associated with strip #7 was homogenized with the polyacrylamide in a minimal volume of PBS, mixed with complete Freund's adjuvant, and injected into rabbits to produce polyclonal anti-heparanase antibodies. This material will also be subjected to amino acid sequencing for the purpose of gene cloning and expression. The anti-heparanase antibodies have been used to immunodetect the enzyme in "Western" blots of fractions eluted from CD-Sephadex (FIG. 5AII(B)) and Con-A Sepharose and in active fractions derived from a biopsy specimen of a human ovarian tumor (FIGS. 7A and 7B).